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PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

"MANUFACTURE BY SPATIALLY SELECTIVE DEPOSITION"

This invention is described in the following statement:-

This invention relates to methods of manufacture by spatially selective deposition of chemical substances.

The spatially selective deposition may be for the purpose of manufacture of solid phase arrays or other physical or chemical reactions such as manufacture of printed circuits, flat panel displays, semiconductor chips, nanotechnology, micro-electromechanical systems, flexible semiconductor circuits, protein chips, lab-on-a-chip microfluidics and DNA chips.

In its broadest form the invention relates to the spatially defined deposition of any of a wide variety of chemical substances onto any predefined surface. Substances may include, but are not limited to, coloured materials, dyes, polymers, catalysts, anti-wetting agents and pigments, etching chemicals, conductors, metals such as gold, layerings and reagents for de-blocking, blocking, protecting and de-protecting agents, derivatisation and activation of solid phase chemical groups. Arrays can include pixel arrays for display panels, deoxyribonucleic acids (DNA), peptides, peptidenucleic acids (PNA), ribonucleic acids (RNA) and other solid phase chemical arrays and arrays assembled by combinatorial chemistry.

The invention will be generally discussed in relation to manufacture of DNA arrays of the type generally known as DNA chips on substrates particularly planar substrates but the invention is not limited to that particular application but has wider ramifications and the invention is not intended to be limited to the manufacture of such DNA chips.

In one form the invention may be said to reside in a method of forming a solid phase array on a substrate, the method including the steps of:

(a) defining at least one region on the substrate by forming an electrostatic charge on that region which is different from the electrostatic charge on other regions of the substrate such as by formation of a latent electrostatic image thereon,

(b) applying an emulsion to the substrate, the emulsion having an electrically charged discontinuous phase and a component to be selectively deposited carried in or comprising the discontinuous phase, and

(c) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electrostatic charge on the region.

The process may further include the step of carrying out repetition of steps (a) to (c) to provide a stepwise deposition process at the same or alternative positions on the substrate and to achieve combinatorial synthesis on the substrate.

In a further form the invention may be said to reside in a method of forming a solid phase array on a substrate, the method including the steps of:

(a) defining at least one region on the substrate by forming an electrostatic charge on that region which is different from the electrostatic charge on other regions of the substrate such as by formation of a latent electrostatic image thereon,

(b) applying an emulsion to the substrate, the emulsion having an electrically charged discontinuous phase and a chemical reagent to participate in forming the solid phase array carried in or comprising the discontinuous phase,

(c) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electrostatic charge on the region and optionally by the use of bias voltage to reduce deposition in non-required regions,

(d) causing a chemical or physical reaction in the at least one region, and

(e) removing the emulsion.

The process may further include the step of carrying out repetition of steps (a) to (e) to provide a stepwise deposition process at the same or alternative positions on the substrate.

Alternatively the process may include a further reaction step where the substrate is "flooded" with a further reagent but that wherein reaction only occurs where the spatially selective deposition has previously occurred.

The electrically charged emulsion may have a negative or a positive charge on it. The electrostatic charge pattern is normally a pattern of electrons but deposition may be to those portions with a negative charge when the emulsion has a positive charge and to those portions without the negative charge where the emulsion is negatively charged. Deposition may be done with the assistance of a bias voltage.

In general the manufacture of DNA chips involves the selective and sequential addition onto a substrate, of molecular units each with a protective group which is removed when the next molecular unit is to be added. One such method of manufacturing DNA arrays uses a process known as the phosphoramidite process which uses a trityl group or derivatives of the trityl group as the protective group, termed a protecting group. The invention is not limited to this process but will be discussed with respect to it.

The phosphoramidite process is a repetitive four stage process (deprotection, coupling, capping and oxidation) for the chemical synthesis of polymers particularly sequences of DNA oligonucleotides to form portions of DNA.

In the phosphoramidite process, a portion of DNA in single stranded form is built up by the sequential addition of one of the four nucleotides (in phosphoramidite form) being the four components which make up DNA, the A, T, G and C nucleotides. Each nucleotide has a chemically removable protecting group on it. A chemical reagent known as a de-protecting agent removes the protecting group exposing a reactive hydroxyl group and in the next stage a nucleotide (in phosphoramidite form) is coupled to the growing DNA string at the reactive hydroxyl group. The next stage is a capping step where any DNA strings which were de-protected but to which a nucleotide was not coupled are permanently capped to prevent unwanted nucleotides from adding to that molecule in later coupling steps. In the final step, oxidation of the newly formed inter-nucleotide phosphite linkage is carried out to convert the linkage to a phosphotriester.

In the manufacture of DNA arrays, a number of different sequence DNA strands are built up on a substrate to enable bio-chemical analysis to take place. In this process it is necessary to selectively de-protect various portions of the array termed features or cells and it is particularly to the requirement for this selective de-protecting that one particular embodiment of substrate of the present invention is directed.

Selective de-protecting by direct light-activated chemistry or photo-removable de-protecting techniques has been developed but these are somewhat inefficient resulting in short solid phase oligodeoxynucleotides in rather large unit feature sizes of 20 to 50 microns and it is an object of this invention to provide a more efficient chemical de-protecting process.

The applicant has surprisingly found that by the use of electrically charged compositions which include the chemical de-protecting agent and which are selectively deposited on predefined areas of a planar or other shaped substrate under the influence of an electric field, then more accurate, localised and efficient de-protection may be possible.

In another form the invention may be said to reside in a method of forming a solid phase chemical array on a substrate using a stepwise reaction process, the method including the steps of:

(f) defining at least one region on the substrate by forming an electrostatic charge on that region which is different from the electrostatic charge on other regions of the substrate such as by formation of a latent electrostatic image thereon,

(g) applying an emulsion to the substrate, the emulsion having the electrically charged discontinuous phase droplets and a chemical reagent carried in or comprising the discontinuous phase,

(h) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electrostatic charge on the region and optionally by the use of a bias voltage to reduce deposition in non-required regions,

(i) causing a chemical reaction in the at least one region,

(j) removing the emulsion, and

(k) carrying out subsequent steps of the stepwise reaction process.

In an alternative embodiment it may be used in a method of forming a DNA array on the substrate using a stepwise coupling process with a chemical de-protecting step prior to each coupling step, the method including the steps of:

(l) preparing a substrate with surface functional groups protected by a removable protecting group;

(m) defining at least one region on the substrate by forming an electric field on that region which is different from the electric field on other regions of the substrate such as by formation of an electro-static image thereon,

(n) applying an emulsion to the substrate, the emulsion having the electrically charged discontinuous phase droplets and a chemical de-protecting reagent carried in the discontinuous phase as discussed above,

(o) attracting the discontinuous phase of the emulsion to the at least one preselected region by attraction by the electric field on the region and optionally by the use of a bias voltage to reduce deposition in non-required regions,

(p) causing chemical de-protecting in the at least one region,

(q) removing the emulsion, and

(r) carrying out subsequent steps of the stepwise coupling process.

The subsequent steps of the stepwise coupling process may be such as those that are carried out in the standard phosphoramidite chemistry for synthesis of oligodeoxynucleotides although as discussed earlier the invention is not limited to this particular chemistry.

It will be realised that the process as discussed above may be repeated a sufficient number of times to synthesise selected oligonucleotides of any sequence and length at least up to 100-mer in a predetermined spatial order, position and feature size on the substrate.

The substrate may be in part insulative or dielectric material which is able to hold an electrostatic charge for sufficient time for attracting the discontinuous phase of the emulsion to the at least one preselected region. The substrate may include dielectrics such as glass, plastics materials or the like and alternatively may include photoconductors such a zinc oxide, selenium and the like.

The step of defining at least one region on the substrate by forming an electrostatic charge on that region may include the step of image reversal to enable deposition in non-charged regions.

The formation of the electrostatic image pattern may be by electrostatic means such as wherein the substrate is a photoconductor and the formation of the electrostatic field is by charging and subsequent discharging by selective illumination. Preferably the illumination may not include radiation in the ultraviolet region as this may cause damage to the DNA molecule. For the assembly of other chemical chips or arrays, however, UV radiation may be used.

In one preferred embodiment, the stepwise coupling process is the phosphoramidite process which uses chemical de-protecting of a trityl group but the invention is not so limited but may include other stepwise coupling or addition processes.

The step of removing the emulsion may include the step of neutralising any residual chemical de-capping agent in the emulsion to prevent it from reacting in non-desired parts of the array.

As discussed above, the emulsion for use in one embodiment of the present invention comprises an electrically insulative continuous phase such as a fluorochemical, an aqueous or a non-aqueous discontinuous phase for instance a hydrocarbon oil which carries the chemical de-capping agent in it in solution, with preferably a surfactant and preferably a charge control agent.

Where the discontinuous phase is water the chemical de-protecting agent may be a weak organic acid such as acetic acid. Acetic acid may be present in a concentration of up to 80% (v/v).

Where the discontinuous phase is a hydrocarbon oil, the chemical de-protecting agent may be a strong protic organic or inorganic acid.

The non-aqueous discontinuous phase which carries the chemical de-protecting agent in solution may be selected from acetone, acetonitrile, cyclohexanone, dibromomethane, dichloromethane (methylene chloride, DCM), trichloromethane, dimethyl formamide (DMF), dioxane, 1,2 dichloroethane (DCE), nitromethane, tetrahydrofuran, toluene, dimethyl formamide or mixtures of compounds such as isopropanol/methylene chloride, nitromethane/methanol, nitromethane/isopropanol, trichloromethane/methanol or isopropanol/methylene chloride. Other hydrocarbons such as decalin and Isopar™ and Norpar™ from Exxon may also be used.

The chemical de-protecting agent may be a Lewis acid or a protonic acid. The Lewis acid may be selected from but not restricted to zinc bromide, titanium tetrachloride, and ceric ammonium nitrate while dilute protonic acids which can be used include, but are not limited to, dilute mineral acids, trichloroacetic acid (TCA), dichloroacetic acid (DCA), benzenesulphonic acid, trifluoroacetic acid (TFA), difluoroacetic acid, perchloric acid, orthophosphoric acid and toluenesulphonic acid. Other acids may include dodecylbenzene sulphonic acid and diphenyl acid phosphate.

An emulsion suitable for the present invention may include a continuous phase, a discontinuous phase which is immiscible in the continuous phase, and a surfactant, the surfactant having a first part which is compatible with the continuous phase and a second part which is compatible with the discontinuous phase, characterised by the continuous phase having a high volume resistivity, the discontinuous phase being electrically charged and the surfactant being selected to not significantly reduce the volume resistivity of the continuous phase.

The term "not significantly reduce the volume resistivity" is intended to mean that the volume resistivity of the continuous phase of the emulsion is not reduced to such an extent that the electrical charge on the discontinuous phase is compromised to the extent that the discontinuous phase will not deposit under the influence of an electric field.

It may be noted, too, that the choice of discontinuous phase or any of its components should be such that it does not significantly partition into the continuous phase and thereby neither significantly reducing the volume resistivity of the continuous phase nor imparting chemical reactivity to the chemical phase.

Preferably the surfactant is one which has a first part which is compatible with the continuous phase and a second part which is compatible with the discontinuous phase.

As discussed above the continuous phase is comprised of a liquid which is electrically insulative and although the characteristics of a particular system would have to be determined empirically it is expected that such a liquid would preferably have a volume resistivity of at least 1×10^6 ohm-cm.

The continuous phase may be selected from hydrocarbons such as hexane, cyclohexane, iso-octane, heptane, aromatic hydrocarbons and isodecane and commercially available mixtures of hydrocarbons such as the Isopars ™ and Norpars™ made by Exxon. The continuous phase may also be selected from fluoro-chemicals including fluoro-carbon compounds. These fluoro-chemicals generally comprise from 2 to 16 carbon atoms and include, but are not limited to, linear, cyclic or polycyclic perfluoroalkanes, bis(perfluoroalkyl)alkenes, perfluoroethers, perfluoroamines, perfluoroalkyl bromides and perfluoroalkyl chlorides such as the Fluorinerts™ made by 3M. The continuous phase may also be selected from silicone fluids such as polyphenylmethyl siloxanes, dimethyl polysiloxanes, polydimethyl siloxanes, cyclic dimethyl siloxanes and the like.

The continuous phase may also be a gel or highly viscous liquid.

The discontinuous phase may be aqueous or non-aqueous. Where the discontinuous phase is non–aqueous it should be immiscible or substantially insoluble in the continuous phase.

The discontinuous phase may be a reagent such as a chemical de-protecting agent or it may be a solvent which carries the active chemical reagent. Alternatively the active chemical reagent may be a solid or insoluble liquid dispersed in the discontinuous phase.

The emulsions according to the invention may also include charge control agents such as ionic or zwitterionic compounds selected from metallic soaps wherein the metals include: barium, calcium, magnesium, strontium, zinc, cadmium, aluminium, gallium, lead, chromium, manganese, iron, nickel, and cobalt and the acid portion is provided by a carboxylic acid of generally but not limited to at least 6 carbon atoms, e.g., caproic acid, octanoic (caprylic) acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linolic acid, erucic acid, tallitic acid, resinic acid, naphthenic acid, succinic acid and the like. Examples of metallic soaps include: aluminium tristearate, aluminium distearate, barium, calcium, lead and zinc stearates; cobalt, manganese, lead and zinc linoleates; aluminium, calcium and cobalt octoates; calcium and cobalt oleates; zinc palmitate; calcium, cobalt, manganese, lead and zinc naphthenates; calcium, cobalt, manganese, lead and zinc resinsates and the like. Other suitable charge directors may include nonpolar liquid soluble ionic or zwitterionic charge director compounds such as sodium dioctylsulfosuccinate, zirconium octoate and metallic soaps such as copper oleate. The charge control agent may also be lecithin and alkyl succinimide. Where the continuous phase is a fluorochemical the charge control agent may include a fluorine analogue of the above compounds.

The additional components in the discontinuous phase may be bio-active agents, reagents and reactants such as acids, bases, derivitisation chemicals, activation agents whether organic or inorganic, pharmaceuticals, dyes, polymers, catalysts and pigments.

An emulsion according to this invention may have a continuous phase which is present in the range of about 40 to 99.99 per cent by volume, a discontinuous phase which is present in a range of from about 0.01 to 60 per cent by volume, optionally a surfactant which is present in a range of about 0.1 to 35 per cent by weight and optionally a charge control agent which is present in a range of 0.01 to 10 per cent by weight.

Throughout this specification the term emulsions is used to indicate emulsions, mini-emulsions and micro-emulsions. Hence the emulsions according to this invention may be true emulsions, that is, emulsions which are formed by the input of mechanical energy such as by shaking, stirring or the like. Alternatively the emulsions may be mini-emulsions which form with the application of more energy than for a standard emulsion. Alternatively the emulsions may be micro-emulsions which form substantially spontaneously provided the correct conditions of temperature and chemical composition are present. Emulsions may have a droplet size of from about 100 microns down to 0.2 microns, mini-emulsions may have a droplet size from 1000 nanometres down to about 50 nanometres and micro-emulsions may have droplet sizes of from about 200 nanometres down to 1 nanometre. It will be noted that there is no hard and fast rule on the size ranges for each. Size ranges will depend upon the composition of each of the phases and the type and quantity surfactant used. The energy applied by the emulsification equipment may also influence size ranges.

For this invention the particles or droplets of emulsion may range in size from 100 microns downwards depending upon the type of emulsion and the applications to which the emulsion is to be applied. Preferably in the case of emulsions for chemical

de-protecting in the phosphoramidite process the emulsions may have a size range from 50 microns down to 20 nanometres.

Where the emulsion of the present invention is to be applied for the chemical de-protecting step of the formation of a DNA array, the invention may be said to reside in a composition being an emulsion including a continuous phase comprising an insulative liquid and a discontinuous phase comprising a non-aqueous or aqueous solvent and a chemical de-protecting reagent in solution in the non-aqueous or aqueous solvent, and wherein the continuous phase has a high volume resistivity and the discontinuous phase is electrically charged.

In an alternative embodiment where the emulsion of the present invention is to be applied for the chemical de-protecting step of the formation of a DNA array, the invention may be said to reside in a composition being an emulsion including a continuous phase comprising an insulative liquid, a discontinuous phase comprising a non-aqueous or aqueous solvent and a chemical de-protecting reagent in solution in the non-aqueous or aqueous solvent and a surfactant, the surfactant having a first part which is compatible with the continuous phase and a second part which includes a group which is compatible with the discontinuous phase, and wherein the continuous phase has a high volume resistivity and the discontinuous phase is electrically charged and the surfactant being selected to not significantly reduce the volume resistivity of the continuous phase.

Preferably where the continuous phase is a fluorochemical the fluorochemical is a perfluoro-carbon such as perfluoro-octanate, linear, cyclic or polycyclic perfluoroalkanes, bis(perfluoroalkyl)alkenes, perfluoroethers, perfluoroamines, perfluoroalkyl bromides and perfluoroalkyl chlorides.

The continuous phase may alternatively be a silicone fluid or an organic liquid, such as a hydrocarbon oil selected from hexane, cyclohexane, iso-octane, heptane, decalin, aromatic hydrocarbons and isodecane and commercially available mixtures of hydrocarbons such as the Isopars ™ and Norpars™ made by Exxon.

Preferably where the continuous phase is a fluorochemical the surfactant is a fluorochemical-hydrocarbon di-block molecule having a fluorophilic part and a lipophilic part. These compounds may also be referred to as amphiphiles. Examples of these are perfluoroaklyl alkanes, perfluorocarbon-propoxypropylene, fluoro-alkyl citrate, perfluoroalkyl-alkylene mono- or di- morpholinophosphate and fluorinated phospholipids, alcohols, polyols or polyhydroxylated or aminated derivatives including amine oxides and amino acid derivatives.

The fluorinated surfactants may also be associated with hydrogenated, non-ionic, anionic, cationic or zwitterionic surfactants. Such hydrogenated surfactants include, for example, phospholipids, copolymers of the polyoxyethylene polyoxyethylenepolyoxypropylene type and polyoxyethylene sorbitan esters.

The surfactants for the emulsion where the discontinuous phase is water may be selected from non-ionic, anionic, cationic or zwitterionic surfactants.

There may also be used combinations of the various surfactants discussed above.

The emulsion according to this form of the invention may further include a charge control agent of the type discussed above. The charge control agent may be sodium dioctylsulfosuccinate, zirconium octoate and metallic soaps such as copper oleate or be lecithin or alkyl succinimide. In some embodiments the function of the charge control agent may be provided by the surfactant.

As discussed above it may be noted that the emulsions according to this embodiment of the invention may be true emulsions or mini-emulsions, that is, emulsions which are formed by the input of mechanical energy such as by shaking, stirring or the like. Alternatively the emulsions may be micro-emulsions which form substantially spontaneously provided the correct conditions of temperature and chemical composition are present.

Alternatively the fluorochemical of this embodiment may be replaced by an alternative compound such as a silicone fluid and hence the surfactant would be selected from a compound which had a silicophilic part.

PROCESS OF MANUFACTURE OF A DNA ARRAY

The process of forming a DNA array according to the present invention may in one embodiment may comprise the following steps:

(a) Substrate. A planar substrate is selected which is in part a dielectric or photoconductor, that is, an electric charge can be formed or impressed to thereby form an electrostatic charge at a selected region or regions or to discharge an electric charge at the selected region or regions.

(b) Substrate preparation. Next binder molecules are covalently bonded onto the substrate. The binder molecules in general have one portion which is covalently joined to the surface of the substrate and a chemically removable portion or chemically de-protectable portion to which linkers carrying a terminal chemically removable portion to which nucleotide in phosphoramidite form are coupled to form the DNA oligonucleotides. The linker molecules have the function of extending the final assembled DNA oligonucleotide (the probe) off the surface of the substrate to thereby provide more efficient access to other test DNA molecules (the targets) such as fluorescent or other detector tagged single stranded DNA molecule families to promote hybridisation which is then followed by analysis of the bound fluorescent or other species directly. There may be some cases in which a DNA array may be built up directly onto a substrate without the use of a binder molecule.

(c) Substrate charging. The substrate has an electrostatic charge placed upon it. A number of known techniques exist for the placement of an electric charge onto a planar substrate such as use of a corona discharge, electron beam gun or by application by a donor roller. The electrical charge may be positive or negative.

(d) Array definition. The substrate is selectively illuminated to discharge the electric charge in a spatially selected array of sites to leave an electrostatic charge pattern. Alternatively the electrical charge may be dissipated on all portions of the substrate except those selected sites. Selective illumination may be by the use of pulsed, modulated, stepped or controlled lasers or various optical techniques such as the use of masks or transparencies and suitable focussing. Preferably the illumination does not include illumination in the short ultra-violet region of the spectrum because such radiation may be harmful to DNA, however, for other forms of array UV may be used. Alternatively a spatially selected array of sites may be selectively charged with or without masks.

(e) De-protection step. The substrate is flooded with a charged liquid emulsion of the present invention which emulsion is insulative in its continuous phase and has a chemical de-protection reagent in solution in its charged discontinuous phase. The reagent is attracted to the charged locations electrostatically and reacts with the chemically removable portion (the protecting group) to remove that portion. The chemical de-protecting reagent is preferably an organic or inorganic acid. The continuous phase of the emulsion is preferably a fluorochemical and the discontinuous phase is preferably an organic solvent. The surfactant is preferably a di-block molecule of a perfluorocarbon and an organic compound in which the perfluorocarbon portion is soluble in the fluorochemical and the organic compound portion is soluble in the organic solvent. After allowing the reaction to occur the emulsion is then washed off with the assistance of a suitable solvent. It is also desirable that before the emulsion is washed of that any de-protection agent remaining in the emulsion is neutralised to prevent it from reacting in non-desired parts of the array.

(f) Nucleotide coupling step. The substrate is then flooded with a reagent including a selected activated nucleotide in phosphoramidite form which becomes chemically coupled to the linker molecules where the chemically removable portion or protecting group has been removed in the previous de-protecting step. The selected nucleotide includes a chemically removable protecting group which protects

the region until the next nucleotide is to be deposited at the region. Excess reagent is then removed.

(g) Capping step. A chemical capping process is used to place permanent caps (eg acetyl groups) onto any linker molecules which were previously de-protected but did not have a nucleotide coupled to them. This is a known step of the phosphoramidite process. Where the nucleotide addition step is expected to be complete or substantially complete this capping step may not be necessary.

(h) Oxidation step. The newly formed phosphite linkage is oxidised to the phosphate (phosphotriester) form to complete one synthetic cycle. This again is a known step of the phosphoramidite process.

(i) Repetition. Steps (c) to (h) are repeated except that the selective illumination may be arranged differently to discharge a different array of regions or to leave charge on the different array of regions and the chemical removal step either removes a protection group from a binder molecule or from a protected previously deposited nucleotide. Repetition is carried on until perhaps 25 nucleotides or more are coupled to form DNA oligomers on a region or regions. This may require up to 100 cycles to ensure that at any one site in any one cycle any one of the four nucleotides A, C, G or T can be coupled. Longer oligomers may be synthesised.

While this solid phase combinatorial chemistry synthesis process has been discussed in relation to the phosphoramidite process it is to be realised that the process is also applicable to other processes which use a stepwise addition process with a chemical capping or de-protecting step or a chemical activation or deactivation step or derivatisation step.

It will be seen generally by this invention that there is provided an arrangement by which spatially selective chemical reactions such as the de-protecting of one or more DNA oligodeoxynucleotides being formed on an array or chip on a substrate is

possible by selective application of the reagent in the discontinuous phase of an emulsion by the process of the present invention.

This then generally describes the invention but to assist with understanding, reference will now be made to a more detailed discussion and examples of the stages of formation of a DNA array.

An experiment was carried out to determine whether a charged emulsion would deposit on an oppositely charged substrate with an electrostatic pattern formed thereon. For this purpose an emulsion was formed with an insulative continuous phase and a discontinuous phase which included an acid and a substrate comprising a zinc oxide photoconductor was charged with a negative pattern. The photoconductor was dip-coated with a solution of Butvar 72 (1% w/v) and the pH indicator methyl orange (at saturation) in cyclohexanone, and dried at 55 degrees C for 30 minutes:

The emulsion comprised the following:

Continuous phase	FC40	0.89ml
Discontinuous phase	5% dichloroacetic acid (w/v) in toluene	0.1ml
	F6H14 (1% in FC40, v/v)	0.01ml

F6H14 is a semifluorinated alkylalkane, 1-(perfluoro-n-hexyl) tetradecane, manufactured by Apollo Scientific Ltd, UK.

It was found that the discontinuous phase deposited only in the area of the charge pattern, giving a pH-dependent colour change from yellow to pink.

This example shows firstly that the novel charged emulsions of the present invention are able to be selectively deposited onto a substrate.

Another application of the present invention may be for the electroless deposition or autocatalytic deposition of metals such as gold copper, cobalt, nickel and palladium.

Electroless deposition may using the electrostatic charged emulsion spatial process of the present invention may be done with either with a substrate which is a photoconductor with a barrier coat (eg. SiO_2) or alternatively the substrate could be glass or a chemically resistant polymer plastic. In the case of glass or plastics the surface would be electrostatically charged through a metal mask and the emulsion containing the catalyst or promoter in the discontinuous phase deposited image wise. An alternative method would be to write to a dielectric substrate surface with a computer controlled electron beam.

The process for the electroless deposition would involve the standard steps for electroless deposition with the exception that the catalyst or chemical used to trigger the electroless deposition would deposited onto the latent electrostatic image using the present invention. Examples of deposition of catalysts would be a palladium activator. Other chemicals that are deposition promoters for various metals are formaldehyde, ethylenediamine, ethylenediamine adducted with 4 moles of propylene oxide, borohydride and amine borane systems. It may be noted that the majority of electroless deposition is done with proprietary electroless solutions from various suppliers either in the electroplating or the semiconductor industries. These companies usually incorporate proprietary ingredients such as wetting agents and additives that for instance control the grain size of the deposited electroless copper. These could also be included in the discontinuous phase of the emulsions of the present invention

Similarly electroless plating is used in the semiconductor industry for the deposition of circuitry and the present invention would be applicable to this technology as well.

This then generally describes the invention but to assist with understanding of the phosphoramidite process and the spatially selective deposition of the present invention reference will now be made to the accompanying drawings which show preferred embodiments of the invention.

In the drawings:

Figure 1 shows the basic principles of the present invention as applied to writing of a DNA chip;

Figure 2 shows the general structure of a DNA chip probe;

Figure 3 shows the various stages of the phosphoramidite process.

Figure 4 shows one method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion;

Figure 5 shows an alternative method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion;

Figure 6 shows detail of the nature of the emulsions of the present invention;

Figure 7 shows detail of the nature of the function of emulsions of the present invention;

Figure 8 shows detail of the nature of an emulsion mediated reaction on the surface of a substrate according to one embodiment of the present invention.

Now looking in more detail to the drawings Figure 1 shows the basic principles of the present invention as applied to writing of a DNA chip.

At stage 1 a substrate is provided which has spacer molecules with a terminal protecting group all over its surface. The entire surface is electrostatically charged negative at stage 2 by the use of a corona discharge. A modulated laser is then used in stage 3 to illuminate those sites where a first deposition is not required. This leaves an array of negatively charged probe cells which are to be derivitised with a first oligonucleotide (eg A). The emulsion according to the invention is then applied to the substrate at stage 4. The emulsion has in its discontinuous phase droplets which are charged positively and include an acid which removes the protecting group on the spacer molecules. The droplets of the discontinuous are drawn to the electrostatically charged probe cells and an acid mediated removal of protecting groups occurs which leaves reactive hydroxyls as shown in stage 5. At stage 6 a reactive amidite is then place on the substrate and reaction occurs where the reactive hydroxyls are present. These reactive amidites each carry a terminal protecting group so that at stage 7 the first cycle is complete and the substrate is then covered

with protecting groups ready for the next oligonucleotide to be deposited in a selected array.

Figure 2 shows the general structure of a DNA chip probe. The substrate has in it a protective layer which include surface derivatisable groups. Onto these surface binder, anchoring and functionalising groups are chemically bonded. From a functional group on these a linker and/or spacer group is joined. Onto the linker/spacer group the DNA is built up as required.

Figure 3 shows the various stages of the phosphoramidite process including that portion which is within the scope of the present invention.

Figure 4 shows one method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion. In this arrangement the substrate is charged with a single point corona discharge device to give a uniform charge pattern on the substrate. A mask is then held over or placed onto the substrate and a light shone onto the mask. Where there are transparent portions in the mask the photoconductor becomes conducting and the charge pattern in those areas is conducted to the conductive layer. This leaves an electrostatic charge pattern in the unexposed areas and it is to these areas that the droplets of the discontinuous phase are attracted.

Figure 5 shows an alternative method by which a substrate according to the present invention may be charged for the deposition of chemicals using an emulsion. In this arrangement a metal or other conducting mask is held over or placed onto the substrate and then the substrate is charged with a single point corona discharge device. This gives a charge pattern on the dielectric layer of the substrate in those areas in which there are apertures in the mask areas and it is to these areas that the droplets of the discontinuous phase are attracted.

Figure 6 shows in detail the various components of an emulsion according to the present invention

Figure 7 shows detail of the nature of the function of emulsions of the present invention as explained in the drawing.

Figure 8 shows detail of the nature of an emulsion mediated reaction on the surface of a substrate according to one embodiment of the present invention as explained on the drawing.

Throughout this specification various indications have been given as to the scope of this invention but the invention is not limited to any one of these but may reside in two or more of these combined together. The examples are given for illustration only and not for limitation.

Throughout this specification and the claims that follow unless the context requires otherwise, the words 'comprise' and 'include' and variations such as 'comprising' and 'including' will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Dated this 30th day of June, 2003.

Raustech Pty Ltd
By its Patent Attorneys
MADDERNS

BASIC PRINCIPLES **AS APPLIED TO DNA CHIP WRITING**

MODIFICATION OF THE PHOSPHORAMIDITE CHEMISTRY FOR SOLID PHASE DNA PROBE SYNTHESIS BY CYCLIC COMBINATORIAL ADDITION OF BASES (A, C, G & T)

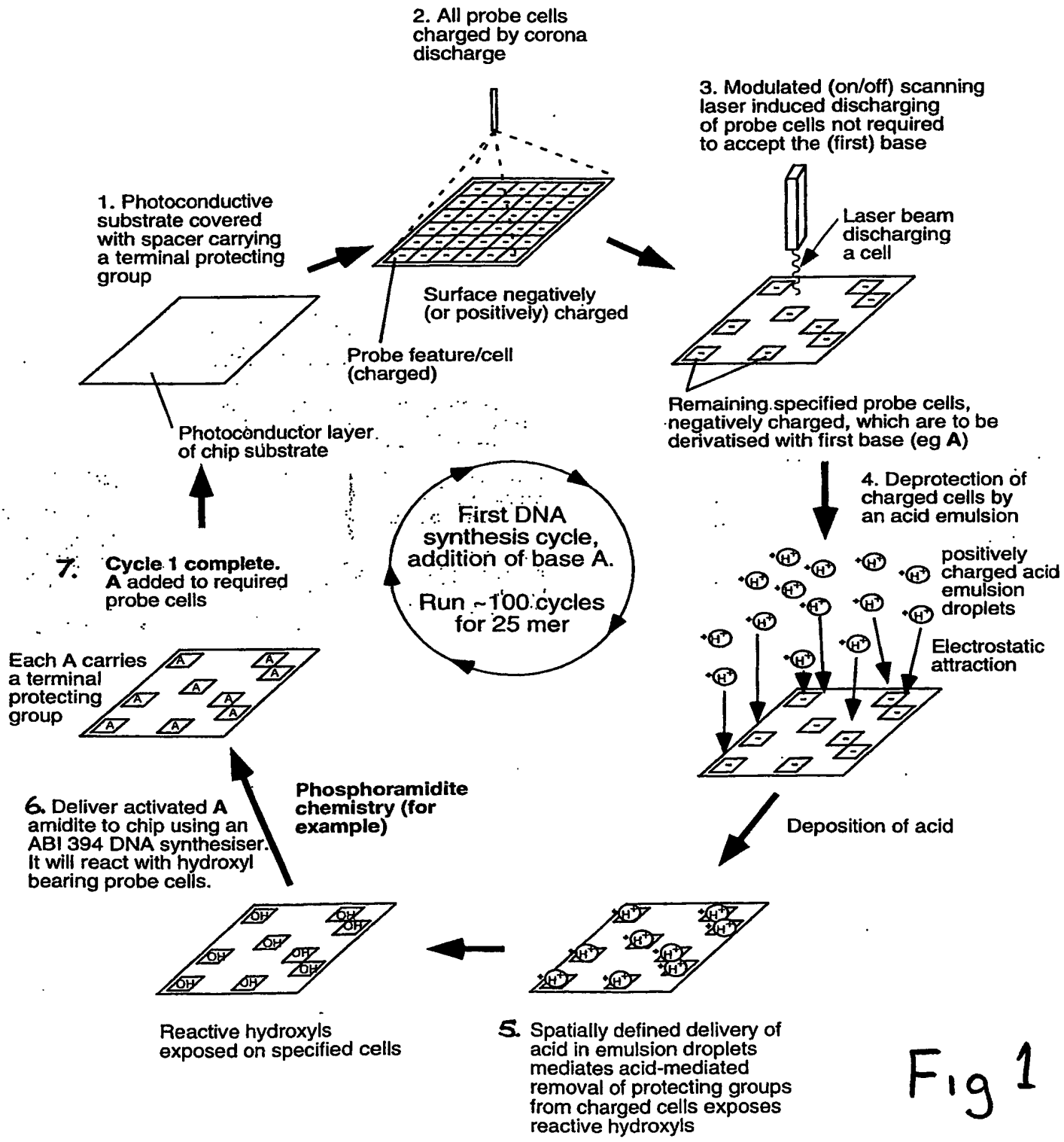


Fig 1

GENERAL STRUCTURE OF DNA CHIP PROBE

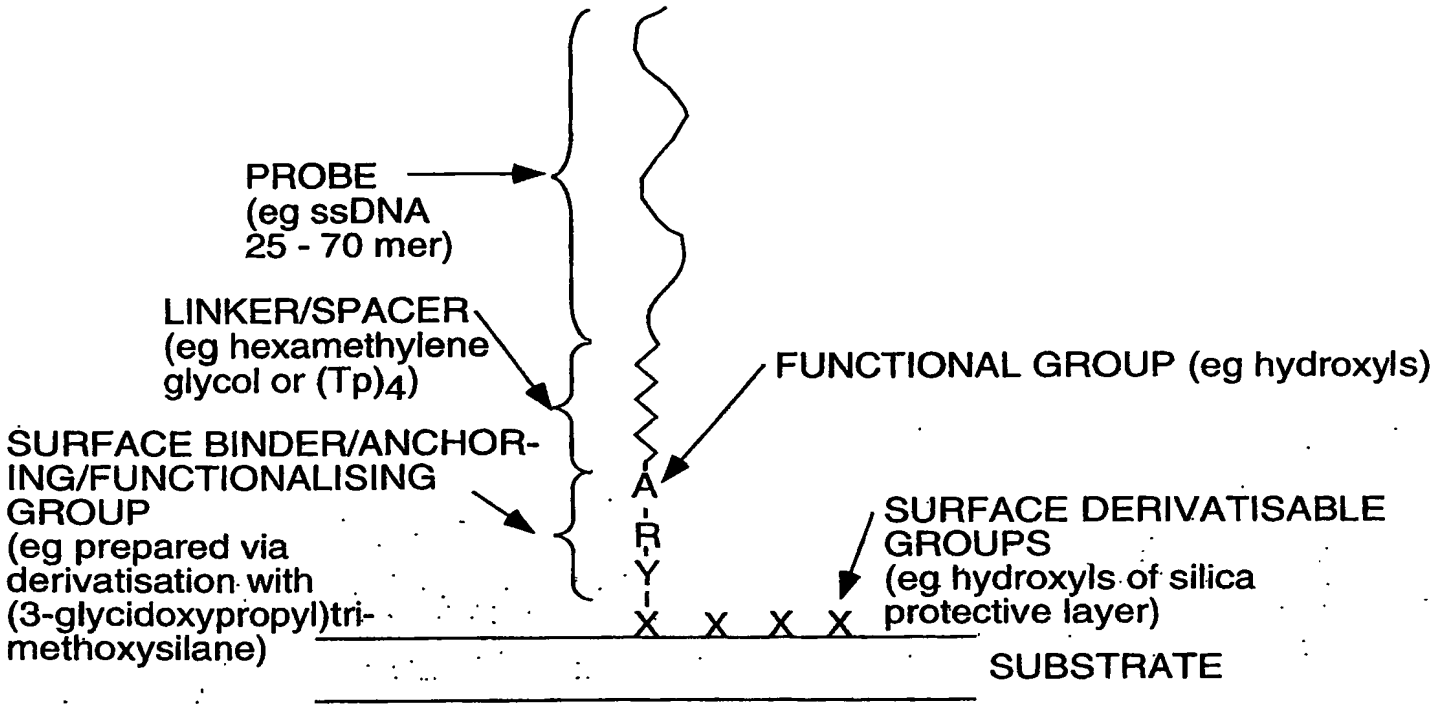


Fig 2

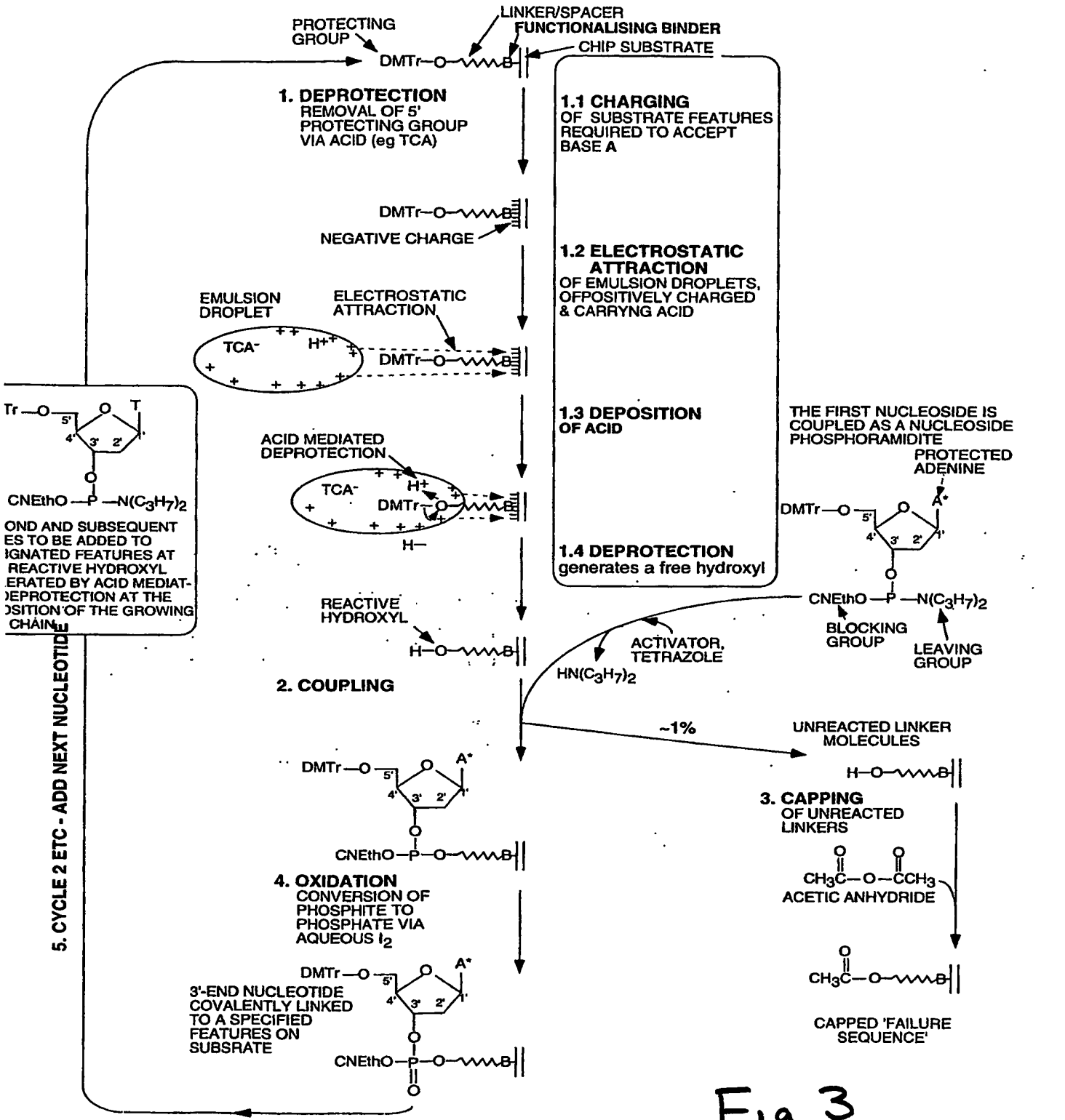

PROTECTING GROUP
LINKER/SPACER
FUNCTIONALISING BINDER
CHIP SUBSTRATE
DMTr—O—B
1. DEPROTECTION
REMOVAL OF 5' PROTECTING GROUP VIA ACID (eg TCA)
1.1 CHARGING
OF SUBSTRATE FEATURES REQUIRED TO ACCEPT BASE A
NEGATIVE CHARGE
1.2 ELECTROSTATIC ATTRACTION
OF EMULSION DROPLETS, OFPOSITIVELY CHARGED & CARRYNG ACID
EMULSION DROPLET
ELECTROSTATIC ATTRACTION
TCA⁻
H⁺
1.3 DEPOSITION
OF ACID
ACID MEDIATED DEPROTECTION
1.4 DEPROTECTION
generates a free hydroxyl
THE FIRST NUCLEOSIDE IS COUPLED AS A NUCLEOSIDE PHOSPHORAMIDITE
PROTECTED ADENINE
CNEthO—P—N(C3H7)2
BLOCKING GROUP
LEAVING GROUP
REACTIVE HYDROXYL
ACTIVATOR, TETRAZOLE
HN(C3H7)2
2. COUPLING
~1%
UNREACTED LINKER MOLECULES
3. CAPPING
OF UNREACTED LINKERS
ACETIC ANHYDRIDE
CAPPED 'FAILURE SEQUENCE'
4. OXIDATION
CONVERSION OF PHOSPHITE TO PHOSPHATE VIA AQUEOUS I2
3'-END NUCLEOTIDE COVALENTLY LINKED TO A SPECIFIED FEATURES ON SUBSRATE
5. CYCLE 2 ETC - ADD NEXT NUCLEOTIDE
OND AND SUBSEQUENT
ES TO BE ADDED TO
IGNATED FEATURES AT
REACTIVE HYDROXYL
ERATED BY ACID MEDIAT-
)EPROTECTION AT THE
)SITION OF THE GROWING
CHAIN

Fig 3

Fluorophor deposition of a mask replica image on a photoconductor strip.
Features comprising a mask replica in charged form were generated by selective light directed discharging through a mask of a completely electron-corona-charged photoconductor, with the fluorophor subsequently deposited thereon via electrostatic attraction of oppositely charged fluorophor-bearing emulsion droplets.

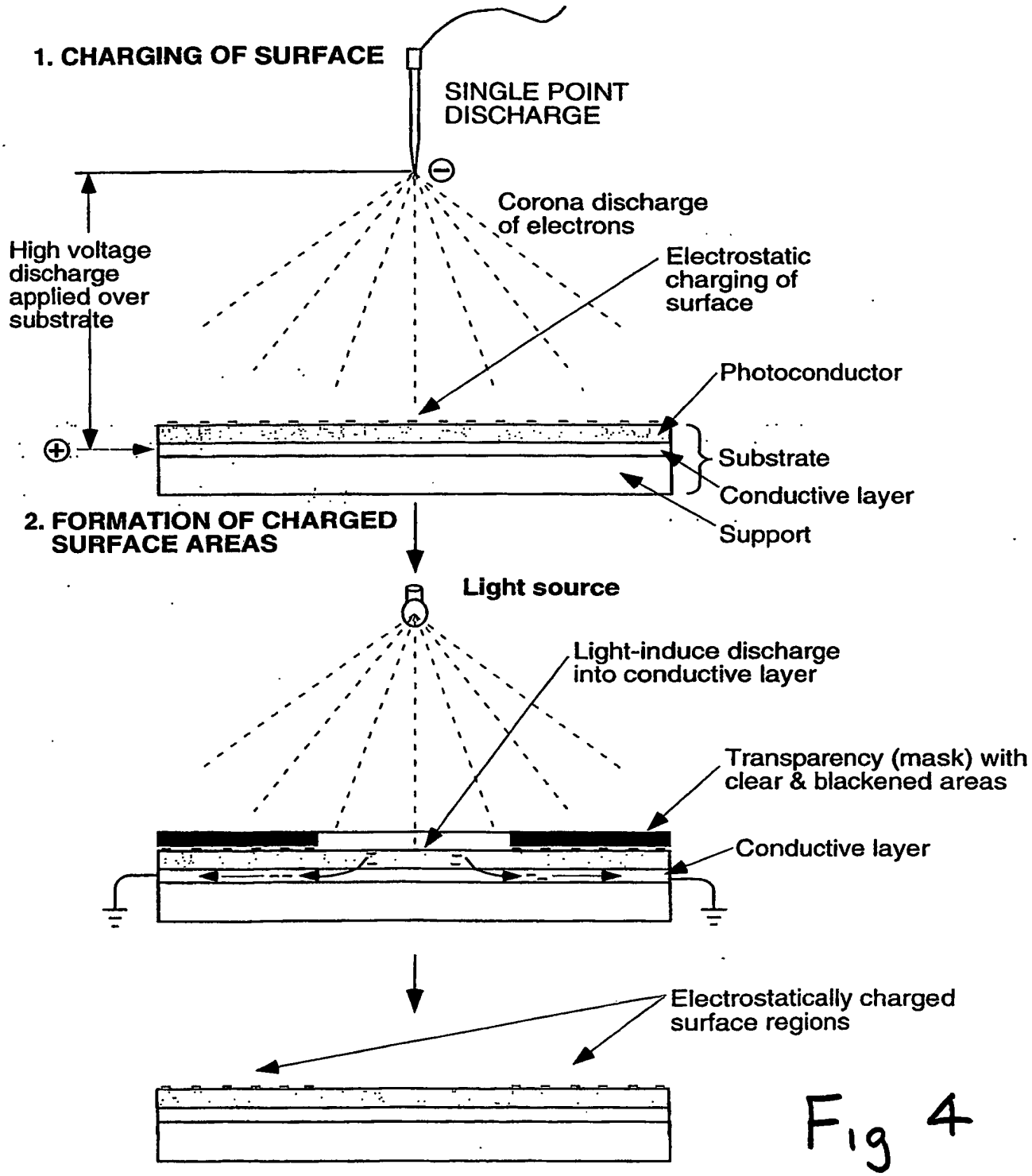


Fig 4

Fluorophor deposition of a mask replica image on a photoconductor strip.
Features comprising a mask replica in charged form were generated by selective light directed discharging through a mask of a completely electron-corona-charged photoconductor, with the fluorophor subsequently deposited thereon via electrostatic attraction of oppositely charged fluorophor-bearing emulsion droplets.

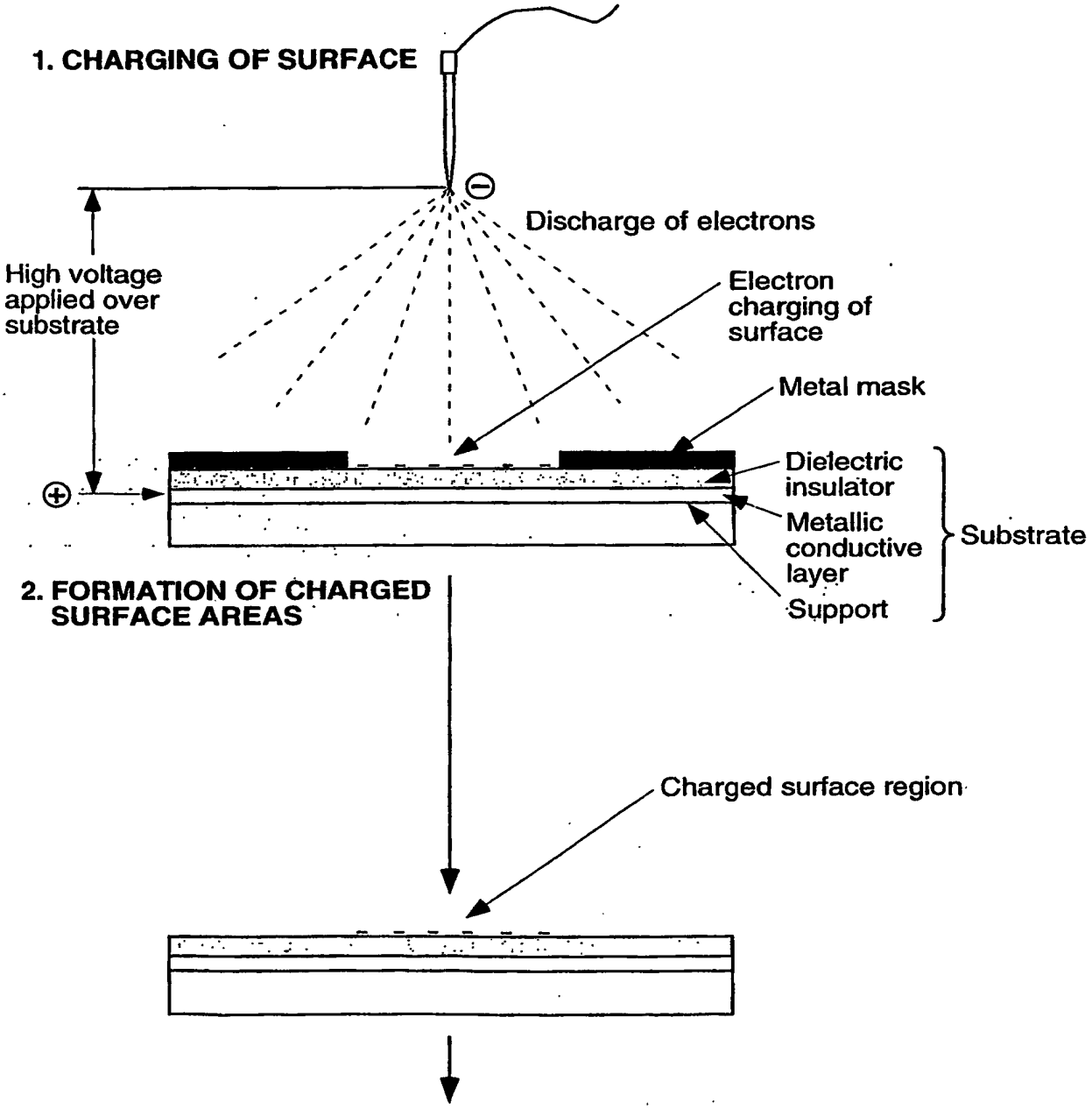
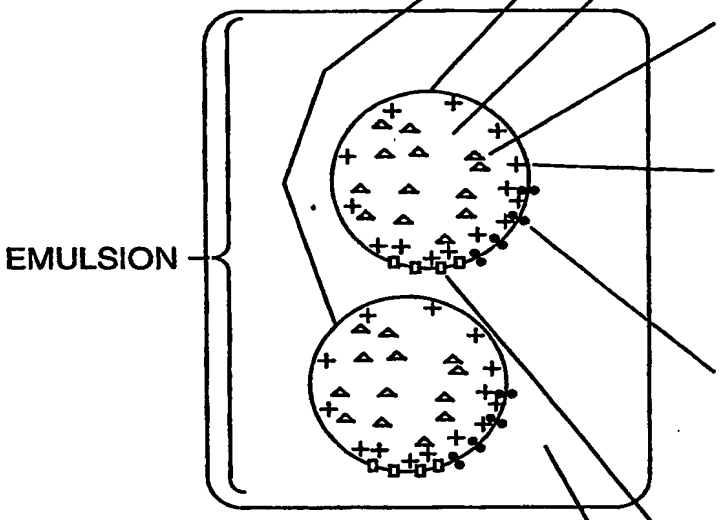


Fig 5

EMULSION DESCRIPTION



COMPONENTS OF AN EMULSION

1. EMULSION DROPLETS

1.1 DISCONTINUOUS PHASE.
To carry chemical substance to be deposited. To be immiscible in the continuous phase

1.2 CHEMICAL SUBSTANCE
To be deposited on a defined surface(s). To be immiscible in the continous phase.

1.3 CHARGE
May be intrinsic to droplets and/or supplied by surfactant and/or charge control agent or induced by an electrostatic field eminating from a charged surface.

1.4 SURFACTANT
To stabilise droplets. May not be required. To have a part compatible with the discontinuous phase and a second part compatible with the continuous phase.
May also impart charge to droplets.

1.5 CHARGE CONTROL AGENT
To impart charge to droplets. Charge can be positive or negative. May not be required.

2. CONTINUOUS PHASE
Electrically insulative

NOTE:

1. DROPLETS WITH SUBSTANCE TO BE DEPOSITED MAY BE INTRINSICALY STABLE AND CHARGED WITH OUT SURFACTANT AND OR CHARGE CONTROL AGENT.

Fig 6

EMULSION FUNCTION

I. EMULSION MEDIATED DEPOSITION OF MATERIAL ON A SURFACE

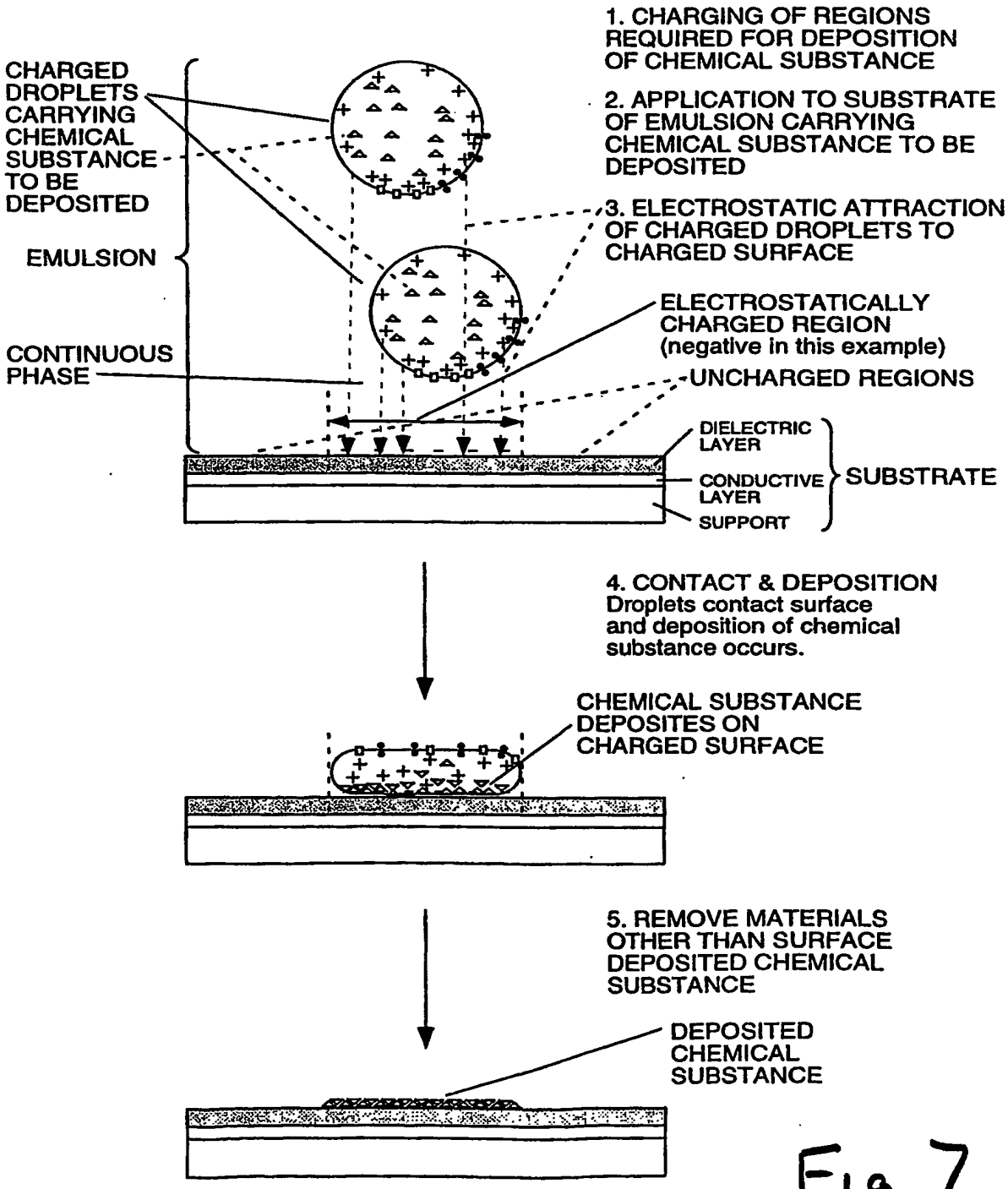


Fig 7

EMULSION FUNCTION

I. EMULSION MEDIATED REACTION ON A SURFACE
eg Acid deprotection of protected linkers on a DNA chip substrate.

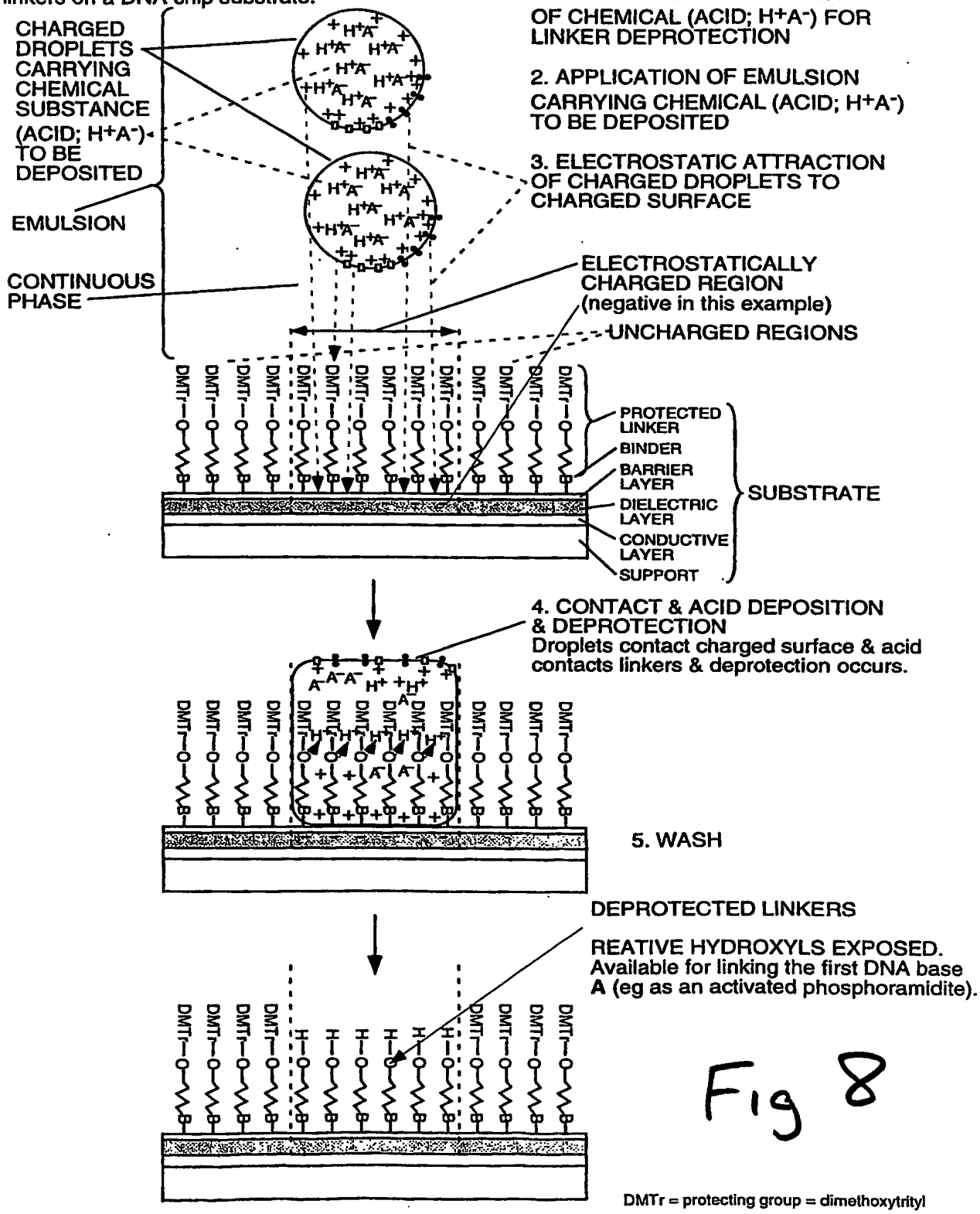


Fig 8

DMTr = protecting group = dimethoxytrityl